Virchows Archiv (1995) 425:569–576

ORIGINAL ARTICLE

Lynne C. Tetlow · Martyn Lees · David E. Woolley

Comparative studies of collagenase and stromelysin-1 expression by rheumatoid synoviocytes in vitro

Received: 26 July 1994 / Accepted: 10 October 1994

Abstract Matrix metalloproteinases such as collagenase and stromelysin are recognised as important cartilage-degrading enzymes in the pathophysiology of rheumatoid arthritis. Synovial fibroblasts and macrophages are the major cellular components of rheumatoid synovium, but the regulation and relative expression of collagenase and stromelysin by these two cell types remains uncertain. Using in vitro cultures of adherent rheumatoid synovial cells we have examined the coordinate or separate expression of collagenase and stromelysin-1 by dual immunolocalisation and Western blotting techniques. Synovial fibroblasts, when activated by macrophage-derived products in primary culture or by interleukin-1/phorbol myristate acetate in subcultures, released significant quantities of collagenase and stromelysin in their inactive, precursor forms. The ratio of released procollagenase: prostromelysin varied between different synovial cell preparations. Dual immunolocalisation studies demonstrated both coordinate and separate expression of the two enzymes by single cells. Approximately 80% of the activated fibroblasts, especially those with stellate morphology, showed co-expression of both enzymes. By contrast synovial macrophages had a modest or negligible capacity to elaborate either enzyme under the same in vitro conditions. In many fibroblastic cells both collagenase and stromelysin were co-localised to the perinuclear Golgi region and the same cytoplasmic compartments. Vesicular structures appear to provide intracellular transport for both enzymes to sites of secretion. Both enzymes showed preferential pericellular binding to a collagenous substratum rather than any association with the plasma membrane/cell surface.

Key words Collagenase · Stromelysin · Regulation Synoviocytes · Immunolocalisation

L.C. Tetlow · M. Lees · D.E. Woolley (✉)
Department of Medicine, University Hospital of South Manchester, West Didsbury, Manchester, M20 8LR, UK

Introduction

The family of matrix metalloproteinases (MMPs) includes the collagenases, stromelysins, gelatinases and matrilysin, enzymes considered to play important roles in connective tissue degradation both in physiological and pathological conditions [2, 16]. The importance of these enzymes in the pathophysiology of arthritic joint destruction is supported by numerous studies of rheumatoid synovial tissue [4, 14, 20, 21, 37, 39]. In vitro studies have shown the metalloproteinases to be secreted from various connective tissue cell types as inactive precursors, requiring activation by limited proteolysis of the N-terminal propeptide [17, 18]. Primary cultures of adherent rheumatoid synovial cells, and subcultured synovial fibroblasts, have been used by various researchers to study the synthesis and regulation of metalloproteinase expression [12, 20, 29, 40]. Such studies have usually provided information on the synthesis and release of specific MMPs, but the regulation of co-ordinate expression of two or more enzymes from individual cells in culture remains poorly understood. Murphy et al. [15] reported that stimulated chondrocyte cultures showed most cells to produce both collagenase and stromelysin, with a few cells producing only one enzyme. MacNaul et al. [12] showed that collagenase and stromelysin were not always coordinately expressed by activated synovial cells, these showing a differential MMP response to specific pro-inflammatory cytokines and phorbol myristate acetate (PMA). Several studies have concluded that different cell types show disparate MMP responses to specific activating agents [8, 13, 35] and others have related changes in cell morphology to the regulation of MMP expression [6, 31, 36, 40].

Primary cultures of adherent rheumatoid synovial cells (ASC) are mainly comprised of fibroblasts, macrophages and a variable proportion of dendritic or stellate cells known to represent prostaglandin E_2 (PGE_2)-activated fibroblasts [6]. These ASC cultures produce large quantities of MMPs and prostaglandins, and the dendritic fibroblasts are recognised as the major source of collage-

nase [40, 41]. Recent studies have shown that subcultured synovial fibroblasts may be stimulated to produce both collagenase and stromelysin [12], gelatinases A and B [31], and stromelysin and gelatinase B [29]. Earlier immunolocalisation studies of cells in culture have noted that immunoreactive collagenase was often confined to perinuclear regions interpreted as Golgi apparatus, and occasionally appeared as fine granular inclusions of the cytoplasm [10, 23, 26, 30, 41]. Similar observations were reported for the immunolocalisation of gelatinase A [24, 27] and gelatinase B [29], but the intracellular packaging and transport mechanisms for each of these enzymes prior to secretion remains uncertain. Since there is evidence that each of the four metalloproteinases may be separately or coordinately expressed, and that each enzyme may respond to different regulatory signals [12, 28, 32, 35], we have examined the production of collagenase and stromelysin in both primary and passaged ASC. We report here that synovial fibroblasts in culture demonstrate both coordinate and separate production of these two metalloproteinases, and that when co-expressed both enzymes appear to be localised within the same cytoplasmic compartments.

Materials and methods

Purification of human interstitial collagenase (pMMP-1) and stromelysin (pMMP-3)

Procollagenase was purified from serum-free conditioned culture medium derived from the breast carcinoma cell line 8701-BC [1], stimulated with 4β-phorbol-12-myristate 13-acetate,25 ng/ml. Procollagenase was purified using sequential fractionation on Green Dye Matrex ion-exchange resin, gelatin-agarose and Heparin-Sepharose affinity chromatography as described previously [11, 19].

Prostromelysin was purified from serum-free conditioned medium derived from rheumatoid synovial fibroblasts stimulated with interleukin-1α (0.5 ng/ml) and tumour necrosis factor-α (30 ng/ml). Purification was achieved by sequential fractionation on S-Sepharose, Green Dye Matrex and Heparin-Sepharose as previously described [11, 19].

Homogeneity of both enzymes was confirmed by silver staining following sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

Antibodies

Sheep antiserum to collagenase was prepared as described previously [38], and rabbit antiserum to stromelysin-1 was prepared using the pure antigen and conventional immunisation procedures. Each antiserum was examined for specificity by Western blotting against each of four MMPs, viz. collagenase, stromelysin-1, gelatinase-A and gelatinase-B [11]. No cross-reactivity was shown and each antiserum reacted only with the precursor and activated forms of the specific immunogen (Fig. 1).

Electrophoresis and Western analysis

SDS-PAGE and Western blot analysis was carried out as described by Okada et al. [19], where samples resolved by SDS-PAGE under non-reducing conditions were electrotransferred onto Zetaprobe GT nylon membrane (BioRad, Richmond, Calif.). The primary antibodies, sheep anti-(human) collagenase and rabbit anti-(human) stromelysin were used at dilutions of 1:500 and 1:1000, respectively. The secondary anti-species antibodies were biotin-conjugated and were used at dilutions of 1:1000, followed by linkage to streptavidin-conjugated horseradish peroxidase (all from Dako, Glostrup, Denmark). Colour development was achieved using the chromogenic substrate 3,3'-diaminobenzidine tetrahydrochloride (DAB) for peroxidase.

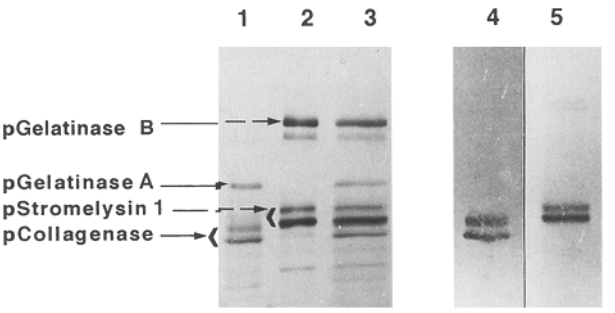

Fig. 1 Demonstration of specificity for the antisera to collagenase and stromelysin using Western blotting. Purified procollagenase and progelatinase A preparations (*lane 1*), prostromelysin and progelatinase B (*lane 2*) and all four enzymes (*lane 3*) were run on SDS-PAGE and stained with silver. The mixture of the four enzymes were also run in *lanes 4 and 5*, electrotransferred onto nylon membrane and probed for collagenase (*lane 4*) and stromelysin (*lane 5*), respectively. No evidence for cross-reactivity was observed

Cell cultures

Rheumatoid synovial tissue obtained at remedial synovectomy or arthroplasty from patients with classic rheumatoid arthritis was enzymically dissociated as described previously [29]. One millilitre of a cell suspension containing approximately 1×10^4 cells ml^{-1} Dulbecco's Modified Eagles' Medium(DMEM) +10% fetal calf serum (FCS, Gibco, Life Technologies Ltd., Paisley, UK) was added to each well of a 12-well culture dish containing a sterile 19-mm-diameter glass coverslip. After 24 h at 37° C in a 5% CO_2:95% air incubator with humidified atmosphere, each well was washed three times with Hanks' balanced salt solution (HBSS, Gibco) to remove the non-adherent cells. The medium was replenished and the ASC were incubated for a further 24 h. Monensin (Sigma, UK) at a final concentration of 3 μM was added for 4 h prior to fixation for immunocytochemical studies. Alternatively, the ASC were grown on coverslips coated with thermally reconstituted collagen as described previously [40].

Subcultures of ASC (passage numbers from 3 to 6) were stimulated with interleukin-1α (IL-1, 0.5 ng/ml; gift from Dr. D.Westmacott, Roche Products,Welwyn Garden City, UK), PMA (25 ng/ml; Sigma, UK) or PGE_2 (1 μg/ml; Sigma, UK) for either 24 or 48 h incubations.

Immunocytochemistry

ASC on glass coverslips were briefly washed twice with HBSS, fixed in 70% ethanol for 5 min, air dried and stored dessicated at 4° C until required. Cells were rehydrated in 50 mM TRIS-buffered saline (TBS) pH 7.4 and examined for metalloproteinases-1 and -3 using primary antisera at dilutions of 1:50 in TBS. Fluorescein isothiocyanate (FITC)-conjugated rabbit anti-sheep IgG (Dako, UK), Texas-red-conjugated donkey anti-rabbit IgG (Amersham, UK) and FITC-conjugated swine anti-rabbit IgG (Dako) were used as secondary antibodies where appropriate and diluted in TBS at concentrations specified by the supplier.

Control incubations with buffer only and similar dilutions of relevant non-immune sera showed no fluorescence. Similarly, no

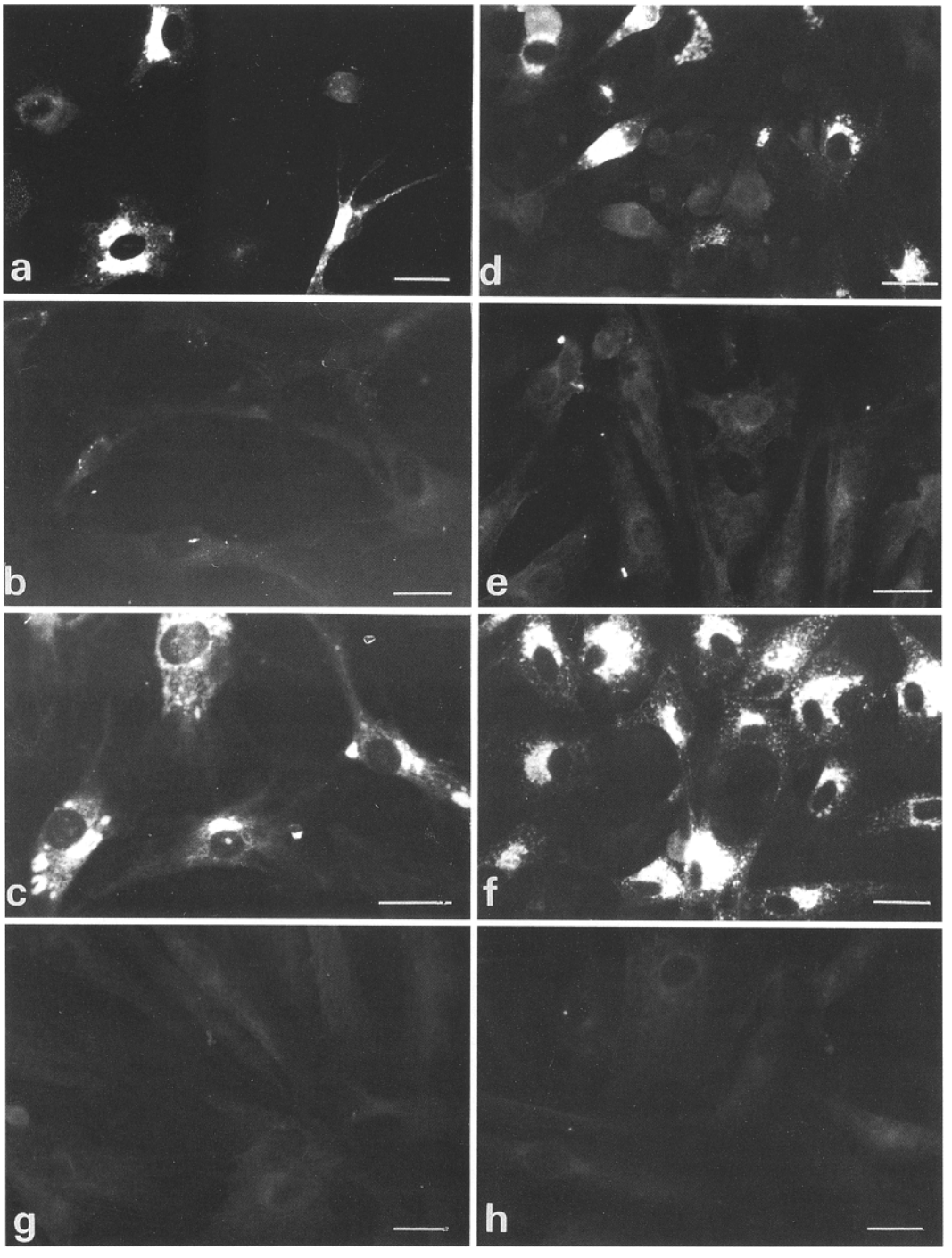

Fig. 2a–h Immunolocalisation of collagenase and stromelysin in primary and subcultured adherent rheumatoid synovial cells (ASC) following addition of monensin. **a** Immunoreactive collagenase demonstrated by FITC in a primary culture of ASC. Juxtanuclear positive staining is pronounced in cells with fibroblastic morphology. Note not all cells are positively stained. **b** Immunoreactivecollagenase localisation by FITC in subcultured (p4) ASC. Note little or no staining for nearly all cells. **c** Subcultured ASC from same preparation as in **b** immunostained for collagenase by FITC following stimulation with interleukin-1 (IL-1) and phorbol myristate acetate (PMA). Nearly all cells are positive and show strong perinuclear staining. **d** Immunoreactive stromelysin demonstrated by FITC in a primary culture of ASC. Note pronounced juxtanuclear staining in cells of fibroblastic rather than those with a rounded, macrophagic morphology. **e** Immunoreactive stromelysin localisation by FITC in subcultured (p4) ASC. Note little or no staining for nearly all cells **f** Subcultured ASC from the same preparation as **e**, stimulated with IL-1 and PMA and subsequently stained for stromelysin. Most cells showed pronounced perinuclear and cytoplasmic FITC staining. **g**, **h** Negative controls. Subcultured ASC stimulated with IL-1 and PMA, and the immunoadsorbed anti-collagenase and anti-stromelysin used as primary antibodies respectively. No positive staining observed. **a–h** *Bar*=10 μm. ×800

positive staining was noted after immunoadsorption of the antibodies (achieved by the addition of 10 μg purified antigen to 500 μl diluted antibody for 1 h at 22° C followed by centrifugation at 2000 *g* and application of the supernatant to the cells).

The cells were examined and photographed using a Zeiss photomicroscope III with filter sets for FITC and Texas-red.

The proportion of cells staining positive for each enzyme was estimated from 8 different fields with X40 objective, and a minimum total of 80 cells per culture being assessed.

Macrophages were identified in the primary cultures using a monoclonal antibody to CD68 (Dako) and a Texas-red-conjugated anti-mouse IgG secondary antibody (Amersham).

Procedures for dual localisation of collagenase (FITC) and stromelysin (Texas-red)

Rabbit anti-stromelysin and sheep anti-collagenase were added together as primary antibodies for 1 h followed, after washing 3×10 min in TBS, by Texas-red-conjugated swine anti-rabbit IgG (1 h) and, after a further 3 washes, by FITC-conjugated rabbit anti-sheep IgG (1 h).

Control incubations to assess problems of cross-reactivity of both primary and secondary antibodies with the various species of immunoglobulin employed were exhaustively tested and found to be satisfactory. Each secondary antibody was shown to be specific for its species IgG, and antigen absorption as described earlier of both primary antibodies eliminated all fluorescence.

Results

Immunolocalisation studies on primary ASC cultures showed that both collagenase and stromelysin were almost exclusively produced by the cells with fibroblastic or stellate morphology (Fig. 2a, d). By contrast, little or no enzyme was demonstrated for the smaller rounded cells shown to be macrophages by the CD68 marker (not shown). Both enzymes were secreted and released by the cells as shown by Western blot analysis of 48 h conditioned medium derived from three primary ASC cultures (Fig. 3). Interestingly each conditioned medium suggested a higher proportion of stromelysin compared to collagenase, but the ratio of these two enzymes appeared to be different for each primary culture, as judged by visual intensity of staining (Fig. 3). Little evidence was found for any processing or activation of the precursor forms of collagenase and stromelysin in these three primary cultures.

Subcultured synovial fibroblasts beyond passage number 4 showed little or no immunocytochemical evidence for collagenase and stromelysin production (Fig. 2b, e), but following stimulation with IL-1α (0.5 ng/ml) and PMA (25 ng/ml) both enzymes were expressed by nearly all the cells (Fig. c, f). This stimulation also increased PGE production, which is known to induce the reversible morphological transformation from a fibroblastic to stellate appearance [6].

Since it was previously reported that the stellate synovial cells were major producers of collagenase in vitro, and released a form that remained bound to a collagen substratum [40], we examined whether this transformed, "activated" fibroblast might also have the ability to directly activate the precursor forms of the two enzymes.

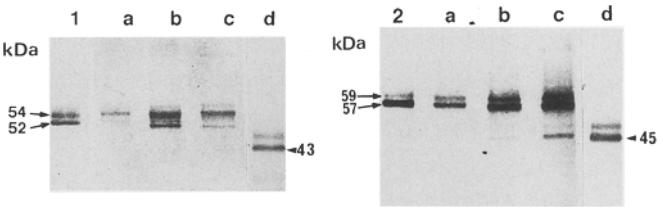

Fig. 3 Western blot analysis of collagenase and stromelysin in conditioned media from three different primary ASC cultures (**a**, **b** and **c**). Evidence for collagenase production and secretion is shown in *1a–c*, and stromelysin production and release in *2a–c*. Purified procollagenase (10 μg/ml), *lane 1*, and purified prostromelysin (10 μg/ml), *lane 2*. Note different staining ratios of collagenase to stromelysin in each of the three samples (*a*, *b* and *c*) and that the precursor forms of each enzyme predominate. *Lanes 1d and 2d* are standards of purified active collagenase and stromelysin respectively, following activation of precursors with 2 mM aminophenylmercuric acetate [11]

Confluent synovial fibroblast cultures were incubated with PMA and PGE_2 for 48 h, during which most of the cells assumed the stellate morphology. The conditioned culture medium from these cultures was subjected to Western blot analysis, which showed a significant increase in collagenase and stromelysin production. However, no evidence was found for the processing or activation of either procollagenase or prostromelysin, even when the cells were incubated with PMA and PGE_2 together with added plasminogen (20 μg/ml) (data not shown). Such observations suggest that the stellate cells were not producing functional plasminogen activator and do not have the capacity to elaborate active collagenase or stromelysin under these in vitro conditions.

Fig. 4a–h Dual immunolocalisation of collagenase and stromelysin in rheumatoid synovial cell cultures. **a** Subcultured ASC (p4) stimulated with IL-1 and PMA, treated with monensin, and immunostained for collagenase with FITC. Note high staining intensity of the stellate cell and the presence of brightly stained compartmental inclusions (*arrows*) along the cytoplasmic extensions of the cell. **b** Same field as in **a** stained for stromelysin with Texas-red. Note similar areas are positively stained and the "inclusions" along the extensions match those positively stained in **a**. **c** Subcultured ASC (p4) stimulated with IL-1 and PMA, treated with monensin, and immunostained for collagenase with FITC. Note intracellular compartments (*arrows*) and vesicular inclusions. **d** Same field as **c** stained for stromelysin with Texas-red. Note intracellular compartments and inclusions similar to those in **c**. **e** Primary culture of adherent synovial cells, monensin treated and subsequently stained for collagenase with FITC. Approximately half the cells have positive staining in perinuclear regions. Note the presence of negative fibroblastic cells (*arrows*). **f** Same field as **e** stained with Texas-red for stromelysin. Note all cells are positive for this enzyme with the three cells shown in **e** preferentially expressing stromelysin (*arrows*). **g** Dendritic cell on a collagen substratum (no monensin) stained for stromelysin with FITC. Note collagen-bound extracellular enzyme associated with the extensions of the cell, and intracellular vesicular staining. **h** Stellate cell from primary ASC culture immunostained for stromelysin with FITC following monensin treatment. Note numerous positive "packages" or vesicles within the cell, and particularly along the cytoplasmic extensions. **a–h** *Bar*=10 μm. ×800. FITC- and Texas-Red-conjugated secondary antibodies were supplied by Dako Ltd., Buckinghamshire, UK and Amersham International plc., Buckinghamshire, UK, respectively

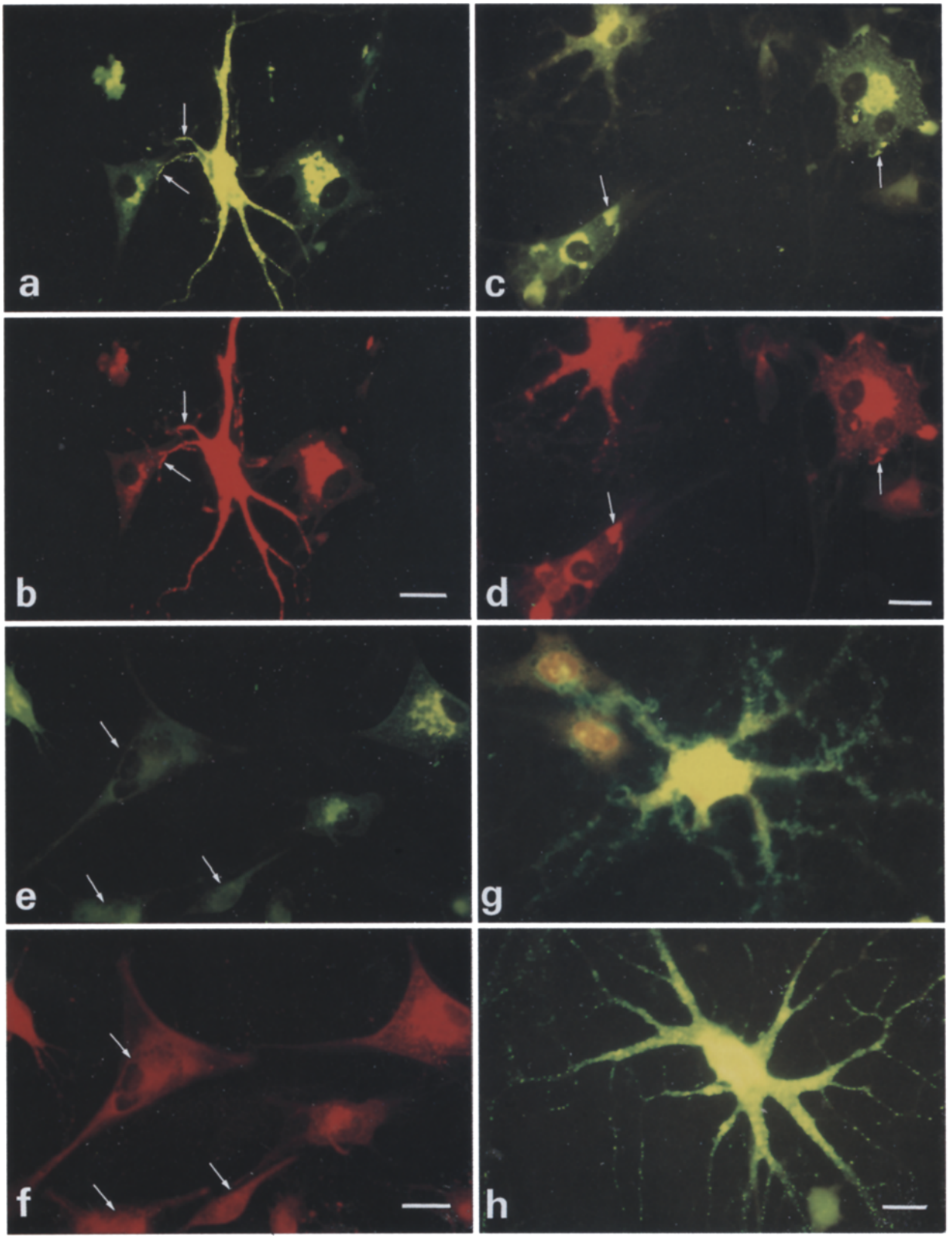
a
c
b
d
e
g
f
h

Table 1 Immunocytochemical summary of collagenase and stromelysin production by synoviocytes in culture (n=8). + to ++++ denotes intensity of juxtanuclear (Golgi) staining. Figures represent the estimated percentage of cells in culture staining positive

	Collagenase		Stromelysin	
Primary culture:				
"Stellate" cells	+++	>80%	++++	>80%
Fibroblasts	++	~50%	+++	~50%
Macrophages	+	<5%	+	<5%
Subcultured cells:				
Fibroblasts	+	<5%	+	<5%
Subcultured cells + IL-1 and PMA:				
"Stellate" cells	++++	>90%	++++	>90%
Fibroblasts	++	>90%	+++	>90%

Dual immunolocalisation studies of collagenase and stromelysin in ASC cultures have shown both the combined and separate expression of both enzymes by individual cells. In general, the ASC cultures produced a more marked response for stromelysin than collagenase, as judged by both localisation and Western blot analysis. Most "activated" cells, either in primary cultures or IL-1/PMA-stimulated subcultures, demonstrated the co-expression of the enzymes, although a small proportion of cells showed a preferential expression of stromelysin (Fig. 4e, f). PMA-induced activation of synovial fibroblasts clearly demonstrated that the stellate morphology was commonly associated with an increased expression of MMPs, immunoreactive enzyme being distributed throughout the Golgi and cytoplasmic extensions (Fig. 4a, b, h). In contrast, the typical fibroblastic morphology usually showed immunoreactive enzyme restricted to a perinuclear Golgi location. Of particular interest was the observation in both cell morphologies of cytoplasmic compartments positively stained for both enzymes, indicating not only that both enzymes were synthesised simultaneously within the Golgi, but also that they may be packaged together for intracellular transport (Fig. 4c, d). Such observations were commonly seen for ASC cultures irrespective of whether or not monensin had been added.

This packaging of the enzymes within cytoplasmic compartments was evident in stellate cells grown either on glass or a collagen substratum. An example of the latter is shown in Fig. 4g, where a stellate cell demonstrates both intracellular and extracellular collagen-bound stromelysin. The latter was occasionally observed as "FITC footprints", where secreting cells had become detached from the collagen during preparation (not shown). This extracellular, collagen-bound stromelysin was usually restricted to the immediate, pericellular location of cells, indicating a strong binding affinity of the enzyme for the collagen substratum, rather than an association with the cell surface. Indeed the consistent observations of a proportion of totally negative cells (e.g. Fig. 4g) in cultures containing high levels of released pMMPs strongly suggests that these enzymes are not effectively bound to the cell surface of synovial fibroblasts or macrophages. The stellate cell (Fig. 4h) shows clear evidence for intracellular packaging of MMP-3 in discrete compartments and vesicles of variable size.

Table 1 provides a summary of collagenase and stromelysin production by ASC and represents immunolocalisation observations derived from eight different synovial cell preparations all treated with monensin prior to fixation. Stellate or dendritic cells were the major source of both collagenase and stromelysin with approximately 80% of all stellate cells in primary cultures showing pronounced staining. Similarly, in subcultured synoviocytes treated with IL-1/PMA almost all cells with the stellate morphology consistently produced a more intense staining for both enzymes. By contrast, only a few macrophagic cells of primary cultures, and non-stimulated synoviocytes of subcultures, showed weak immunolocalisation for collagenase and stromelysin. Primary ASC cultures not treated with monensin (n=10) showed qualitative similarities, but quantitatively less staining.

Discussion

This study has demonstrated that rheumatoid synovial fibroblasts, when activated by macrophage-derived products in primary cultures or by IL-1/PMA in subcultures, produce significant quantities of collagenase and stromelysin. Most of the activated fibroblasts, and especially those with the stellate or dendritic morphology, showed dual expression of these enzymes. Moreover, in many cells both collagenase and stromelysin were co-localised to the same cytoplasmic compartments, providing evidence that intracellular transport, and presumably secretion, may be an integrated event for these two enzymes. However, a minority of the positively stained cells (<10%) showed the expression of only one enzyme, usually stromelysin. Thus, this study indicates that rheumatoid synovial fibroblasts subjected to activation in vitro may express collagenase and stromelysin either coordinately or separately. Our observations suggest that MMP transport within the cell is achieved via enzyme "packages", especially along cytoplasmic extensions to sites of secretion, thereby allowing deposition of MMPs at tissue sites remote from the main body of the cell. However, further ultrastructural studies are needed to provide conclusive evidence for this.

An ultrastructural immunolocalisation study of procollagenase in human gingival fibroblasts demonstrated procollagenase in membrane-coated vesicles at the Golgi complex, and transportation via large extended vesicles to the surface of the cells [30]. The present study is in general agreement with those findings, but provides evidence that both procollagenase and prostromelysin are packaged within the same cytoplasmic compartments. Such observations lend weight to the concept, based on biochemical studies, that active stromelysin is a requirement for the complete activation of the collagenase precursor [9, 17, 18]. The packaging and secretion of both

enzymes appears to be a common feature of IL-1/PMA-activated synovial fibroblasts, but it is uncertain whether this response is similar for other fibroblast-activating factors.

Unique patterns of MMP synthesis have been observed in many studies, suggesting that each enzyme is subject to specific regulatory signals and has distinct extracellular functions. [3, 8, 12, 22, 25, 35]. This concept is supported by numerous reports which demonstrate specific biological control mechanisms for the regulation of both individual and coordinate MMP expression [7, 22, 27–29, 35]. Monocyte-macrophages have been shown to have a different capacity for MMP expression than fibroblasts [29, 35] – observations supported by this study, which shows the synovial macrophages to have a modest capacity to elaborate collagenase and stromelysin compared to synovial fibroblasts under identical in vitro conditions.

Kinetic studies using fibroblast cultures have shown that collagenase is rapidly released from the cells after biosynthesis and post-translational processing, without any significant intracellular storage [2, 33]. The monovalent ionophore monensin has been used in several studies to prevent secretion and to demonstrate an intracellular accumulation of collagenase and other metalloproteinases [10, 24]. Such studies have indicated that the rate of enzyme secretion under physiological conditions probably reflects de novo synthesis and not intracellular storage. Our cell culture studies were performed both with and without the addition of monensin. Although the latter results in an increased intracellular distribution for both enzymes, no qualitative differences were observed for the intracellular localisation. Thus the vesicular structures and their content were not a consequence of monensin effects.

Secretion of collagenase by synovial fibroblasts in culture was previously shown to reflect a uniform release of enzyme across the whole plasma membrane, as judged by collagenase "footprints" of similar FITC staining intensity on a collagen substratum [40]. Similar observations were seen for the release of stromelysin by synovial fibroblasts, the enzyme appearing to bind to the collagen substratum underlying the cells. Trabandt et al. [30] concluded from their ultrastructural studies that collagenase released from extracellular vesicles became associated with the plasma membrane of the gingival fibroblasts. We have little evidence from our studies that this occurs for synovial fibroblasts. ASC cultures in the absence of monensin seldom showed immunoreactive enzyme for the cell surface even though high levels of both enzymes were present in the culture medium.

Western blot analyses have clearly demonstrated that different preparations of primary ASC, and activated fibroblasts from subcultures, produce different quantitative ratios of collagenase and stromelysin. Whereas PMA activation of subcultured fibroblasts produced a more equal ratio of the two enzymes, primary cultures usually showed a greater quantity of stromelysin compared to collagenase. As yet we do not know whether the compartmental content of the two enzymes reflects the enzyme ratio monitored extracellularly. Since a small proportion of cells within a single culture may show preferential production of either collagenase or stromelysin alone, it would seem that a defined quantitative relationship of collagenase and stromelysin production is unlikely. Moreover, this latter observation also confirms that the antibodies used in the dual localisation study were not showing cross-reactivity.

Our observations support the concept that "activated" synovial fibroblasts, especially those with stellate morphology, produce both collagenase and stromelysin in a coordinate manner. Moreover, both enzymes appear to be packaged within the same cytoplasmic compartments and smaller vesicles which presumably permit intracellular transport to sites of secretion. Both enzymes remained in the precursor form after secretion, even in the presence of added plasminogen, indicating that even stellate synovial fibroblasts were unable to activate or process the MMP precursors under these in vitro conditions. Similarly, no precursor processing was observed in primary ASC cultures which contained plasminogen derived from FCS, and macrophages which are known to produce plasminogen activator [34]. Whether this reflects an excess of secreted inhibitors (either tissue inhibitors of metalloproteinases or inhibitors of plasminogen activator) remains to be examined. Lastly, both enzymes showed preferential binding to a collagenous substratum rather than an association with the plasma membrane or cell surface.

Since both collagenase and stromelysin-1 have been demonstrated by immunolocalisation and in situ hybridisation in freshly excised rheumatoid tissues [7, 14, 29], including sites of cartilage erosion [39], our observations strongly suggest that the synovial fibroblast is potentially the major source of both enzymes. By contrast if these in vitro observations can be extrapolated to in vivo cell behaviour, it would appear that the macrophage has the potential to produce relatively little collagenase or stromelysin-1, but its ability to express the proinflammatory cytokines tumour necrosis factor-α and IL-1 [5] would be of major importance for the induction of these two metalloproteinases by synovial fibroblasts.

Acknowledgements This work was supported by research grant WO147 from the Arthritis and Rheumatism council, UK. We thank consultant orthopaedic surgeons H. Bertfield (Wythenshawe Hospital, Manchester), T. Dunningham (Tameside Hospital, Manchester) and M. Morris (Devonshire Royal Hospital, Buxton) for the supply of rheumatoid tissues.

References

1. Alessandro R, Minafra S, Pucci-Minafra I, Onisto M, Garbisa S, Melchiori A, Tetlow L, Woolley DE (1993) Metalloproteinase and TIMP expression by the human breast carcinoma cell line 8701-BC. Int J Cancer 55:250–255
2. Birkedal-Hansen H, Moore WGI, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA (1993) Matrix metalloproteinases: a review. Crit Rev Oral Biol Med 4:197–250
3. Brown CC, Hembry RM, Reynolds JJ (1989) Immunolocalization of metalloproteinases and their inhibitors in rabbit growth plate. J Bone Joint Surg [Am] 71:580–593

4. Case JP, Lafyatis R, Remmers EF, Kumkumian GK, Wilder RL (1989) Transin/stromelysin expression in rheumatoid synovium. Am J Pathol 135:1055–1064
5. Chu CQ, Field M, Allard S, Abney E, Feldmann M, Maini RN (1992) Detection of cytokines at the cartilage/pannus junction in patients with rheumatoid arthritis: implications for the role of cytokines in cartilage destruction and repair. Br J Rheumatol 31:653–661
6. Gadher SJ, Woolley DE (1987) Comparative studies of adherent rheumatoid synovial cells in primary culture: characterisation of the dendritic (stellate) cell. Rheumatol Int 7:13–22
7. Gravellese EM, Darling JM, Ladd AL, Katz JN, Glimcher LH (1991) In situ hybridization studies of stromelysin and collagenase messenger RNA expression in rheumatoid synovium. Arthritis Rheum 34:1076–1084
8. Hanemaaijer R, Koolwijk P, Le Clerck L, De Vree WJA, Van Hinsbergh VWM (1993) Regulation of metalloproteinase expression in human vein and microvascular endothelial cells. Biochem J 296:803–809
9. He C, Wilhelm SM, Pentland AP, Marmer BL, Grant GA, Eisen AZ, Goldberg GI (1989) Tissue cooperation in a proteolytic cascade activating human interstitial collagenase. Proc Natl Acad Sci USA 86:2632–2636
10. Hembry RM, Murphy G, Cawston TE, Dingle JT, Reynolds JJ (1986) Characterization of a specific antiserum for mammalian collagenase from several species: immunolocalization of collagenase in rabbit chondrocytes and uterus. J Cell Sci 81:105–123
11. Lees M, Taylor DJ, Woolley DE (1994) Mast cell proteinases activate precursor forms of collagenase (pMMP-1) and stromelysin (pMMP-3), but not gelatinases A and B (pMMP-2 and pMMP-9). Eur J Biochem 223:171–177
12. MacNaul KL, Chartrain N, Lark M, Tocci J, Hutchinson NI (1990) Discoordinate expression of stromelysin, collagenase and tissue inhibitor of metalloproteinases-1 in human synovial fibroblasts. J Biol Chem 265:17238–17245
13. Mauviel A (1993) Cytokine regulation of metalloproteinase gene expression. J Cell Biochem 53:288–295
14. McCachren SS (1991) Expression of metalloproteinase inhibitor in human arthritic synovium. Arthritis Rheum 34:1085–1093
15. Murphy G, Hembry RM, Reynolds JJ (1986) Characterisation of a specific antiserum to rabbit stromelysin and demonstration of the synthesis of collagenase and stromelysin by stimulated rabbit articular chondrocytes. Collagen Relat Res 6:351–364
16. Murphy G, Docherty AJP, Hembry RM, Reynolds JJ (1991) Metalloproteinases and tissue damage. Br J Rheumatol 30:25–31
17. Nagase H, Enghild JJ, Suzuki K, Salvesen G (1990) Stepwise activation of the precursor of matrix metalloproteinase 3 (stromelysin) by proteinases and (4-aminophenyl) mercuric acetate. Biochemistry 29:5783–5789
18. Nagase H, Ogata Y, Suzuki K, Enghild JJ, Salvesen G (1991) Substrate specificities and activation mechanisms of matrix metalloproteinases. Biochem Soc Trans 19:715–718
19. Okada Y, Nagase H, Harris ED Jr (1986) A metalloproteinase from human rheumatoid synovial fibroblasts that digests connective tissue matrix components. Purification and characterization. J Biol Chem 261:14245–14255
20. Okada Y, Takeuchi N, Tomita K, Nakanishi I, Nagase H (1989) Immunolocalisation of matrix metalloproteinase 3 (stromelysin) in rheumatoid synovioblasts (B cells): correlation with rheumatoid arthritis. Ann Rheum Dis 48:645–653
21. Okada Y, Gonoji Y, Nahanishi I, Nagase H, Hayakawa T (1991) Immunohistochemical demonstration of collagenase and tissue inhibitor of metalloproteinase (TIMP) in synovial lining cells of rheumatoid synovium. Virchows Arch [B] 59:305–312
22. Overall CM, Wrana JL, Sodek J (1989) Independent regulation of collagenase, 72-kDa progelatinase, and metalloendoproteinase inhibitor expression in human fibroblasts by transforming growth factor-β. J Biol Chem 264:1860–1869
23. Reddick ME, Bauer EA, Eisen AZ (1974) Immunocytochemical localization of collagenase in human skin and fibroblasts in monolayer culture. J Invest Dermatol 62:361–366
24. Reynolds JJ, Hembry RM (1992) Immunolocalization of metalloproteinases and TIMP in normal and pathological tissues. Matrix [Suppl] 1:375–382
25. Saarialho-Kere UK, Kovacs SO, Pentland AP, Olerud JE, Welgus HG, Parks WC (1993) Cell-matrix interactions modulate interstitial collagenase expression by human keratinocytes actively involved in wound healing. J Clin Invest 92:2858–2864
26. Sakamoto M, Sakamoto S (1984) Immunocytochemical localization of collagenase in isolated mouse bone cells. Biomed Res 5:39–46
27. Salamonsen LA, Nagase H, Suzuki R, Woolley DE (1993) Production of matrix metalloproteinase 1 (interstitial collagenase) and matrix metalloproteinase 2 (gelatinase A: 72 kDa gelatinase) by ovine endometrial cells in vitro: different regulation and preferential expression by stromal fibroblasts. J Reprod Fertil 98:583–589
28. Shapiro SD, Campbell EJ, Senior RM, Welgus HG (1991) Proteinases secreted by human mononuclear phagocytes. J Rheumatol 18 [Suppl 27] 95–98
29. Tetlow LC, Lees M, Ogata Y, Nagase H, Woolley DE (1993) Differential expression of gelatinase B (MMP-9) and stromelysin-1 (MMP-3) by rheumatoid synovial cells in vitro and in vivo. Rheumatol Int 13:53–59
30. Trabandt A, Gay RE, Birkedal-Hansen H, Gay S (1990) Intracellular secretory pathway and ultrastructural localization of interstitial procollagenase in human gingival fibroblasts. Matrix 10:339–347
31. Unemori EN, Werb Z (1986) Reorganization of polymerized actin: a possible trigger for induction of procollagenase in fibroblasts cultured in and on collagen gels. J Cell Biol 103:1021–1031
32. Unemori EN, Hibbs MS, Amento EP (1991) Constitutive expression of a 92 kDa gelatinase (type V collagenase) by rheumatoid synovial fibroblasts and its induction in normal human fibroblasts by inflammatory cytokines. J Clin Invest 88:1656–1662
33. Valle K-J, Bauer EA (1979) Biosynthesis of collagenase by human skin fibroblasts in monolayer culture. J Biol Chem 254:10115–10122
34. Vassalli J-D, Wohlwend A, Belin D (1992) Urokinase-catalyzed plasminogen activation at the monocyte/macrophage cell surface: a localized and regulated proteolytic system. (Current topics in microbiology and immunology 181) Springer, Berlin Heidelberg, pp 65–73
35. Welgus HG, Campbell EJ, Cury JD, Eisen AZ, Senior RM, Wilhelm SM, Goldberg GI (1990) Neutral metalloproteinases produced by human mononuclear phagocytes. J Clin Invest 86:1496–1502
36. Werb Z, Hembry RM, Murphy G, Aggeler J (1986) Commitment to expression of the metalloendopeptidases, collagenase and stromelysin: relationship of inducing events to changes in skeletal architecture. J Cell Biol 102:697–702
37. Wolfe GC, MacNaul KL, Beuchel FF, McDonnell J, Hoerrner LA, Lark MW, Moore VL, Hutchinson NI (1993) Differential in vivo expression of collagenase messenger RNA in synovium and cartilage. Arthritis Rheum 36:1540–1547
38. Woolley DE, Crossley MJ, Evanson JM (1976) Antibody to rheumatoid synovial collagenase. Eur J Biochem 69:421–428
39. Woolley DE, Crossley MJ, Evanson JM (1977) Collagenase at sites of cartilage erosion in the rheumatoid joint. Arthritis Rheum 20:1231–1239
40. Woolley DE, Brinckerhoff CE, Mainardi CL, Vater CA, Evanson JM (1979) Collagenase production by rheumatoid synovial cells: morphological and immunohistochemical studies on the dendritic cell. Ann Rheum Dis 38:262–270
41. Woolley DE, Tetlow LC, Evanson JM (1980) Collagenase immunolocalisation studies of rheumatoid and malignant tissues. In: Collagenase in normal and pathological connective tissues. Wolley DE, Evanson JM (eds) Wiley, Chichester, pp 105–125